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Association of *hsp70-2* and *hsp-hom* gene polymorphisms with risk of acute high-altitude illness in a Chinese population

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Abstract High-altitude illness (HAI) is a potentially fatal condition involving genetic and environmental components. Accumulated experimental evidence suggests that heat shock proteins (Hsps), especially HSP70, can protect cells and organs against different types of damage. We investigated whether genetic variation in constitutive and inducible *hsp70* genes could be associated with risk of HAI. The association between polymorphisms of the *HSP70* family genes and risk of HAI was determined in 56 patients with HAI and in 100 matched controls by genotyping for the polymorphisms +190 G/C, +1267 A/G, 2437 G/C in the *hsp70-1, hsp70-2,* and *hsp70-hom* genes by using polymerase chain reaction–restriction fragment length polymorphism. The data showed that there was no statistically significant difference in the genotype and allele distributions of *hsp70-1,* in *hsp70-2* allele and *hsp70-2 A/A and A/B* genotypes, and in allele distribution of *hsp70-hom* among patients with HAI and controls (χ^2 test, $P > 0.05$). However, there was a significantly higher frequency of *hsp70-2* B/B and *hsp70-hom* A/A and B/B genotypes and a significantly lower frequency of the *hsp70-hom* A/B genotype in the HAI patients compared with the controls ($P < 0.05$ for all). The risk associated with the *hsp70-2* B/B and *hsp70-hom* A/A, A/B, and B/B genotypes were 4.017 (95% CI = 1.496–10.781; $P = 0.004$), 2.434 (95% CI = 1.184–5.003; $P = 0.012$), 0.299 (95% CI = 0.148–0.602, $P = 0.001$), and 5.880 (95% CI =1.145–30.196, $P = 0.026$), respectively. Our results suggest that individuals with *hsp70-2* B/B and *hsp70-hom* A/B and B/B genotypes may be more susceptible to HAI, whereas those with *hsp70-hom* A/B genotype may be tolerant to HAI. Further studies in individuals of different age and sex are warranted to elucidate the underlying mechanisms of this association and the possible functions of different genotypes of *hsp70-2* and *hsp70-hom* under hypoxic stress.

INTRODUCTION

High-altitude illness (HAI) is a collective term for acute mountain sickness, the less frequent but potentially fatal high-altitude cerebral edema, and high-altitude pulmonary edema occurring in persons exposed to high altitude (Basnyat and Murdoch 2003). Many people in the world are exposed to high altitudes (more than 2500 m) either as residents, travelers, workers, or military immigrants. The most important risk factors include rate of ascent, altitude reached, individual susceptibility as well as history of HAI and of permanent residence at greater than 900 m, exertion, age, sex, and disease states, especially respiratory-tract infections (Honigman et al 1993). Although the exact mechanism causing HAI is unknown, the basic pathophysiological change is hypoxemia caused by hypoxia and the consequent vascular changes in the whole body, especially in the brain and lungs (Hackett 1999; Roach and Hackett 2001).

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Some individuals are more susceptible to HAI than others; therefore, it is important to explain differences in susceptibility in order to develop methods to predict the risk. There are still limited and somewhat controversial data about genetic polymorphisms and related susceptibility to HAI. For example, endothelial nitric oxide synthase gene polymorphisms were associated with susceptibility to high-altitude pulmonary edema in Japan (Droma et al 2002) but not in Europe (Weiss et al 2003); single-nucleotide polymorphisms (SNPs) of the endothelin-1 (the potent hypoxia-inducible factor [HIF]–targeted vasoconstrictor) gene also differ in the Andeans compared with low-altitude populations (Moore et al 2004). Various biochemical mediators such as nitric oxide (NO), endothelin-1, and the renin-angiotensin-aldosterone system and possible oxygen-sensing mechanisms may be involved in hypoxic adaptation such as HIF-1, and individuals who have had high-altitude pulmonary edema once run an unpredictable but significant risk of recurrence; therefore, there are constitutional or genetic components in the etiology of HAI (Woods and Montgomery 2001; Mortimer et al 2004). Studies have investigated the possible involvement in physiological adaptation to hypoxia of several genes such as angiotensin-1–converting enzyme, tyrosine hydroxylase, serotonin transporter, and endothelial NO synthase (Woods and Montgomery 2001). As yet, there is no firm association between any identified genetic polymorphism and HAI and high-altitude pulmonary edema (Dehnert et al 2002). Such genetic variations could provide a possible mechanism to explain interindividual variation in response to hypoxia and enhanced or reduced tolerance to high altitude.

Heat shock proteins (Hsps) are inducible conserved proteins (Craig et al 1993; Morimoto et al 1994) whose expression is triggered when organisms are exposed to heat shock or to a variety of other stress stimuli, including hypoxia, ischemia, and oxidative free radicals (Xiao et al 2002, 2003). HSPs are usually grouped into several general families (HSP110, HSP90, HSP/HSC70, HSP60, HSP47, and the small HSPs [HSP10–30]) on the basis of their apparent molecular masses in sodium dodecyl sulfate polyacrylamide gels. The primary biological function of HSPs is to fulfill chaperone activity (Craig et al 1993; Hartl 1996). As a dominant chaperone, HSP70 plays an important role in the assembly and transport of newly synthesized proteins within cells, as well as in the removal of denatured proteins (Hightower 1991; Hartl 1996; Kiang and Tsokos 1998). The increased expression of HSP70 can protect the heart, brain, kidney, and lung from stressful injury (Currie et al 1993; Hutter et al 1994; Marber et al 1995; Plumier et al 1995, 1997; Radford et al 1996; Suzuki et al 1997; Yenari et al 1998; Rajdev et al 2000; Okubo et al 2001). Furthermore, HSP70 protein and its antibody have been identified as being involved in the pathogenesis of hypertension, atherosclerosis, coronary heart disease, acute heat-induced illness, and stroke (Pockley et al 1998, 2003; Chan et al 1999; Gromadzka et al 2001; Wu et al 2001; Jin et al 2004a, 2004b). Therefore, it is not unexpected that polymorphisms in the *hsp* genes may contribute to differential disease susceptibility because these proteins are involved in stress tolerance (Favatier et al 1997; Martin et al 2004; Wu et al 2004). The human HSP70 family consists of 3 main genes: *hsp70-1, hsp70-2,* and *hsp70-hom* (Milner and Campbell 1990). Both *hsp70-1* and *hsp70-2* encode the similar heat-inducible protein Hsp70, but *hsp70-1* is also constitutively expressed at a low level, whereas *hsp70-hom* encodes a non–heat-inducible protein that shares high homology with the protein products of *hsp70-1/2.* These genes are polymorphic, potentially accounting for variation in their functions and susceptibility to stress tolerance (Wu et al 2004). To date, a few studies reported possible associations of SNP in the *hsp70* genes with autoimmune diseases (Pugliese et al 1992; Jarjour et al 1996; Favatier et al 1997; Fraile et al 1998; Vargas-Alarcón et al 2002), Parkinson's disease (Wu et al 2004), abacavir hypersensitivity (Martin et al 2004), and lung cancer (Rusin et al 2004), but there was no apparent association of a *hsp70-1* promoter polymorphism +110 C/A with risk of myocardial infarction, body mass index, or any coronary disease traits (Bolla et al 1998).

On the basis of previous work on the functions of Hsp proteins in stress responses, we explored the possibility that there might be an association between *hsp70* polymorphisms and susceptibility to HAI, a stress response to low oxygen. Using the polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP) method, we investigated the associations of 3 known polymorphisms (+190 G/C and +1267 A/G in the 5′ untranslated regions of the *hsp70-1* and *hsp70-2* genes, respectively, and 2437 G/C in the coding region of the *hsp70-hom* gene) with the risk of HAI in 56 male patients and 100 healthy male controls 20 to 23 years of age, who lived in the same environment for at least 1 year, and experienced the same conditions of immigration from low (400 m) to a high sea level (2600–2800 m) within 2 days.

MATERIALS AND METHODS

Subjects

This study was conducted in August of 2001 in Sichuan province. All individuals were of Chinese Han ethnicity, 20 to 23 years of age, and lived in the same environment for at least 1 year. Hundreds of men came to Chengdu (capital of Sichuan province) to receive the same training for at least 1 year from Sichuan, Henan, and Shaxi provinces and Chongqing Specialized Economic Zone, where

Table 1 Characteristics of the patients with HAI and healthy controls[a]

Groups	Patients with HAI	Healthy controls	*P* value[b]
Number of subjects	56	100	
Age (mean ± SD, y)	19.6 ± 1.1	20.1 ± 1.5	>0.05
Time for stay in Chengdu (mean ± SD, y)	1.01 ± 0.9	1.12 ± 0.8	>0.05
Height (mean ± SD, cm)	172.8 ± 1.7	174.3 ± 1.5	>0.05
Weight (mean ± SD, kg)	60.7 ± 4.3	61.8 ± 5.4	>0.05
Ratio of smoking (%)	25.4	25.8	>0.05

[a] HAI, high-altitude illness; Hsp, heat shock protein.
[b] Two-sided *t* or χ^2 tests for difference between the patients and controls.

they lived at sea levels around 200–800 m. They then moved from Chengdu to the border between Sichuan province and Tibet Specialized Administration Zone where the sea level is around 2600–2800 m, at the same rate of ascent with the same physical activity. The selected individuals were divided into HAI and control groups according to whether or not they had experienced episodes of HAI during this training movement. The disease group consisted of 56 young male patients with HAI, whereas the control group comprised 100 male individuals with similar ages but without symptoms of HAI. The patients were diagnosed according to the Chinese Medical Association criteria (Wu 1995), mainly depending on symptoms and signs such as headache, anorexia, nausea, vomiting, fatigue, dizziness, and sleep disturbance and excluded other disorders such as exhaustion, dehydration, hyperthermia, alcohol hangover, migraine, and heat-induced illness. There were no HAI patients with high-altitude cerebral edema or high-altitude pulmonary edema in these individuals. Personal information on sociodemographics, family history of diseases, living environments, and lifestyle factors were obtained by a standardized questionnaire administered in person by 2 research investigators. Approximately 2 mL fresh blood was drawn by vein puncture from all participants. Written informed consent was obtained from all participants after a full explanation of the study. The Ethics Committee of Tongji Medical College and Chengdu Wujing Hospital approved this study.

Genotyping of *HSP70* polymorphisms

DNA was isolated from 300 μL blood using a commercial DNA extraction kit according to the manufacturer's instructions (Gentra Corp, Minneapolis, MN, USA). Genotypes for polymorphisms +190 G/C in the *hsp70-1* gene, +1267 A/G in the *hsp70-2* gene, and +2437 G/C in the *hsp70-hom* gene were determined by previously described PCR-based assays using the primers described in Vargas-Alarcón et al (2002). In brief, a PCR reaction was carried out in a 25-μL volume containing 500 ng of genomic DNA, 200 μmol/L dNTPs deoxyribonucleotide trisphosphates; 2 mM $MgCl_2$; 1× *Taq* DNA polymerase buffer; 1 μmol/L each primer; and 1 unit of *Taq* DNA polymerase (Fermentas Inc, Hanover, MD, USA). The following PCR protocol was used for amplifying *hsp70* gene: initial amplification by incubating the PCR mixture at 94°C for 5 minutes followed by 35 cycles of incubation at 94°C and corresponding anneal temperatures (57°C for *hsp70-1* and 56°C for *hsp*70-2 and *hsp70-hom*) for 1 minute each; 72°C for 1 minute; and a final incubation at 72°C for 10 minutes. For RFLP detection, the amplified PCR fragments of *hsp70-1, hsp70-2,* and *hsp70-hom* gene were digested with restriction enzymes *Bsr*BI, *Pst*I, and *Nco*I (Fermentas) respectively. Subsequently, the digested products of *hsp70-1* gene were analyzed on 12% polyacrylamide gel and those of *hsp70-2* and *hsp70-hom* gene were separated on 1.5% agarose gels. These gels were stained with ethidium bromide (0.5 μg/mL), and genotypes and alleles were determined by analysis of different bands described in Vargas-Alarcón et al (2002). All analyses were carried out blindly to the patient's disease status.

Statistical analysis

Measurements of continuous data were analyzed by univariate analysis of variance and Student's *t*-tests. Qualitative data were computed by the Pearson χ^2 contingency tables. Genotype and allele frequencies for each polymorphic site were calculated, and the differences between the patients and controls were tested by the χ^2 test. The strata without significant differences in genotypes were combined as the reference group for the analysis of HSP70 genotypes. Adjusted odds ratios (ORs) with 95% confidence intervals (CI) were computed to test the magnitude of associations between HAI risk and the genotypes by multivariate logistic regression analysis. All calculations were performed using the SPSS statistical package (SPSS for Windows 12.0, SPSS Inc., Chicago, IL, USA).

RESULTS

Characteristics of the subjects

Basal characteristics of the patient and control group are shown in Table 1. This table shows that patients and con-

Table 2 Frequencies distributions of the genotypes and alleles of the *hsp70-1*, *hsp70-2*, and *hsp70-hom* in patients with HAI and healthy controls[a]

		Patients with HAI (n = 56)		Healthy controls (n = 100)		
Gene		**Number**	**%**	**Number**	**%**	***P* value***
hsp70-1						
Genotype	b1/b1	4	7.1	9	9.0	>0.05
	b1/b2	44	78.6	81	81.0	
	b2/b2	8	14.3	10	10.0	
Alleles	b1	52	46.4	99	49.5	>0.05
hsp70-2						
Genotype	A/A	13	23.2	25	25.0	<0.01
	A/B	30	53.6	68	68.0	
	B/B	13	23.2	7	7.0	
Alleles	B	56	50.0	82	41.0	>0.05
hsp70-hom						
Genotype	A/A	22	39.3	21	21.0	<0.01
	A/B	28	50.0	77	77.0	
	B/B	6	10.7	2	2.0	
Alleles	B	40	35.7	81	40.5	>0.05

[a] HAI, high-altitude illness; *hsp*, heat shock protein gene.
* Chi-square test for difference in the distributions of the genotypes and alleles between the patients and controls.

trols were very similar in the distributions of age, time of stay in Chengdu, height, weight, and smoking status ($P > 0.05$ for all).

Distribution of the HSP70 genotypes and alleles in HAI patients and controls

The distributions of the *hsp70-1*, *hsp70-2*, and *hsp70-hom* genotypes and alleles in HAI patients and in controls are listed in Table 2. There was no significant difference in the distributions of both the variant genotypes ($P = 0.689$) and alleles ($P = 0.638$) of the *hsp70-1* gene between the patients and controls, although the *hsp70-1* b2/b2 genotype was slightly but not significantly higher among the patients (14.3%) than among the controls (10.0%) ($P = 0.422$) (Table 2). For the *hsp70-2* gene, the frequency of the *hsp70-2* B/B genotype was significantly higher among the patients (23.2%) than among the controls (7.0%) ($P < 0.01$), but there was no significant difference in the allele frequency distributions (Table 2). For the *hsp70-hom* gene, the frequencies of the *hsp70-hom* A/A and B/B(c2) genotypes were higher in the patients than in the control, whereas the frequency of the *hsp70-hom* A/B genotype was lower in the patients. The differences in the genotype distribution were all statistically significant ($P < 0.01$) (Table 2). However, there was no significant difference in the frequency of the *hsp70-hom* variant B allele between the patients and the controls (Table 2).

The association of the genotypes with the risk of HAI

Finally, we analyzed the association of all *hsp70* genotypes and alleles with risk of HAI (Tables 3 and 4). As shown in Table 3, *hsp70-2* B/B and *hsp70-hom* B/B genotypes were associated with significantly increased risk of HAI (OR = 4.02 [95% CI = 1.50–10.8] and 5.88 [95% CI = 1.15–30.2], respectively), compared with *hsp70-2* A/A, *hsp70-hom* A/A genotypes. In contrast, the *hsp70-hom* A/B genotype was associated with a significantly decreased risk of HAI (OR = 0.35; 95% CI = 0.15–0.60). However, there was no significant association of 3 variant *hsp*70 alleles with risk of HAI (Table 4).

DISCUSSION

Previous studies suggest that Hsp70 can protect different organs from oxidative damages resulting from ischemia/reperfusion and may play an autoprotective role in asthma and lung injury (Bonay et al 1994; Wong and Wispe 1997; Bertorelli et al 1998; Tong and Luo 2002). The HSP70 family proteins may be the most predominant and particularly interesting group of proteins involved in the major histocompatibility complex in disease susceptibility (Favatier et al 1997). The basic pathophysiological changes of HAI are hypoxemia caused by hypoxia and the consequent vascular changes of whole body, especially in brain and lungs. There is genetic susceptibility to HAI but it is unknown whether there is an association between genetic variation in HSPs genes and risk of HAI. Using HAI patients and controls well matched on ascent, altitude reached, other risk factors including history of HAI and permanent residence (lower than 900 m), exertion, age, sex, disease states (especially respiratory-tract infection), our results show for the first time that the variant *hsp70-1* b1/b2 and b2/b2 genotypes are not associated

Table 3 Regression analysis for risk of HAI associated with the genotypes of the *hsp70-1*, *hsp70-2*, and *hsp70-hom*[a]

Genotype	HAI cases/healthy controls (number)	OR (95% CI)*	*P* value*
hsp70-1			
b1/b1	4/9	1.00	
b1/b2	44/81	1.22 (0.38–1.94)	0.133
b2/b2	8/10	1.80 (0.55–4.05)	0.646
b1/b1 + b1/b2	48/90	1.00	
b2/b2	8/10	1.50 (0.55–4.05)	0.646
hsp70-2			
A/A	13/25	1.00	
A/B	30/68	0.85 (0.42–1.96)	0.074
B/B	13/7	3.57 (1.50–10.8)	0.004
A/A + A/B	43/93	1.00	
B/B	13/7	4.02 (1.50–10.8)	0.004
hsp70-hom			
A/A	22/21	1.00	
A/B	28/77	0.35 (0.15–0.60)	0.012
B/B	6/2	2.86 (1.15–30.2)	0.001
A/A + A/B	50/98	1.00	
B/B	6/2	5.88 (1.15–30.2)	0.034

[a] CI, confidence interval; HAI, high-altitude illness; OR, odds ratio; *hsp*, heat shock protein gene.
* Obtained from the logistical regression with adjustment for OR (95% CI).

Table 4 Regression analysis for risk of HAI associated HSP70 variant alleles[a]

Gene/allele		HAI patients (n = 112)	Controls (n = 200)	OD (95% CI)	*P* value
hsp70-1	b1	52	99	0.884 (0.556–1.405)	0.603
	b2	60	101	1.131 (0.712–1.798)	0.603
hsp70-2	A	56	118	0.695 (0.436–1.107)	0.125
	B	56	82	1.439 (0.904–2.292)	0.125
hsp70-hom	A	72	119	1.225 (0.759–1.977)	0.405
	B	40	81	0.816 (0.506–1.317)	0.405

[a] CI, confidence interval; HAI, high-altitude illness; OD, odds ratio; *hsp*, heat shock protein gene.

with increased risk for HAI. However, the *hsp70-2* B/B and *hsp70-hom* B/B genotypes are more often found in HAI patients and are associated with an increased risk of HAI. Conversely, the *hsp70-hom* A/B genotype was associated with a decreased risk of HAI. These findings are biologically plausible and consistent with some previous reports. For example, no association was found between myocardial infarction and coronary risk traits in the *hsp70-1* promoter polymorphism +110 C/A (Bolla et al 1998).

An interaction between environmental and genetic predisposing factors is thought to be involved in the etiology of HAI (Mortimer et al 2004); however, most of these interactions, including the cytoprotective HSP70 with many effective protection molecules such as adenosine triphosphate–sensitive potassium channels, reactive oxygen species, NO, protein kinases to chronic hypoxia (Kolar and Ostadal 2004) are not yet known. HSP expression is under a complex regulatory control operating at both transcriptional and translational levels (Morimoto 1998; Shi et al 1998). This expression depends on the activation of heat shock transcriptional factor–1, constitutively present as a monomer that trimerizes and binds to the heat shock elements of heat shock genes on heat activation. The 2 highly conserved *hsp70-1* and *hsp70-2* genes share a similar heat shock element, which suggests that they have similar affinities for heat shock factors (Milner and Campbell 1990, 1992). Whether the polymorphisms in the *hsp70* genes have any biological or functional significance is unclear, although we did not observe any confounding bias in our findings by many factors affecting the occurrence of HAI. Finally, given this negative finding, it is not known whether the *hsp*-1 polymorphisms are involved in the susceptibility to HAI in Chinese Han populations. Further work will be needed to investigate the influence of these polymorphisms on the regulation of *hsp70-1* and their possible interaction with other important polymorphisms or genes related to HAI.

Nevertheless, our data show a significant and interesting association of *hsp70-2* B/B and *hsp70-hom* B/B genotypes with increased risk of HAI and an association of *hsp-hom* A/B with a decreased risk of HAI. Our study

sample size is relatively small but the subjects are well matched. These findings suggest a potential role of the *hsp70-2* and *hsp70-hom* genes, especially *hsp70-hom,* in susceptibility to HAI in the Chinese Han population. There are only a few investigations regarding the possible role of *hsp70-2* in some diseases; for example, Bougacha-Elleuch et al (2000) indicated that there was lack of association between *hsp-2* polymorphism and autoimmune thyroid diseases. Clarimon et al (2003) found that the severity of behavioral disturbances in patients with Alzheimer's disease carrying 1 or 2 *hsp70-2* deletion alleles was higher for those patients carrying the *hsp70-2* insertion. Fekete et al (2003) reported that the *hsp70-2* GG genetic variation was associated with low inducibility of HSP70 and with the increased risk of acute renal failure in very low birth weight neonates. It is unknown whether there is a lower inducibility of Hsp70 in HAI patients carrying the *hsp70-2* B/B genotype and if a lower level of Hsp70 would be insufficient to protect these individuals from high-altitude stress.

The *hsp70-hom* polymorphic (*Nco*I) site at nucleotide 2437, which corresponds to a Met to Thr amino acid substitution at position 493 (Milner and Campbell 1990). Although few studies investigated possible roles of the *hsp70-hom* polymorphisms, there are several possible explanations for our findings of positive association between these genotypes and HAI. According to the Pociot's theory (Pociot et al 1993), the neutrality of the Thr residue (T allele) may affect the efficiency of the *hsp70-hom* protein in acting as a molecular chaperone by lowering the strength of hydrophobic interactions between the chaperone and target protein. In a proposed structural model of peptide binding based on the model of the HLA class I molecule, the altered amino acid at position 493 (Met to Thr) is on one β sheet, which forms the floor of the peptide-binding groove affecting specificity and activities (Pociot et al 1993). The *hsp70-hom* polymorphic C allele translated to a Met residue, a hydrophilic amino acid. Such alteration may affect interaction of the HSP70 molecular chaperone with hydrophobic proteins and consequently impair its ability to assemble and transport some newly synthesized proteins within cells, as well as to remove denatured proteins that may form as a result of damage like in acute myocardial infarction. On the other hand, the *hsp70-hom* gene located in the class III region of the HLA complex and the genes of the class III region display strong linkage disequilibrium with the HLA class I and II genes. However, it is unknown whether the association between the *hsp70-hom* gene and HAI may be a result of closely linked polymorphisms elsewhere within the HLA region. Further studies are needed to identify other polymorphic sites within the HLA region that may be associated with the *hsp70* polymorphisms observed in HAI patients. Finally, the protein encoded by *hsp70-hom* is expressed under normal conditions albeit at a low level and is not stress inducible. Interestingly, Vargas-Alarcón et al (2002) reported a significant association of the *hsp70-hom* +2437T allele with spondyloarthropathies in Mexicans, and Martin et al (2004) reported that the presence of the *HLA*-B*5701 and *hsp70-hom* 493T alleles are necessary for the development of abacavir hypersensitivity. Thus, although the significance of basal expression level of *hsp70-hom* and its possible interaction with other proteins remain unknown, our data suggest that the conserved *hsp70-hom* gene may be very important in HAI disease susceptibility.

In summary, the present data suggest that genetic variation in the *hsp70-2* and *hsp70-hom* genes may contribute to the susceptibility to HAI. Further investigations with larger groups are warranted to confirm the significance of our findings and functionality of these observed polymorphisms.

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